

Amendments to the Claims

Please amend Claims 1, 11, 20, 21, 25, 26, 30-32, 36 and 37. The Claim Listing below will replace all prior versions of the claims in the application:

Claim Listing

1. (Currently amended) An assay for screening a test substance for ~~HIF~~ hypothalamic inhibitory factor (HIF) inhibitory activity of an ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$, comprising the steps of:
 - (a) contacting liposomes containing, or cells expressing or containing, the ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ with the test substance in the presence of a compound comprising isotopic Rb^+ , under suitable conditions for isotopic Rb^+ uptake by the liposomes or the cells;
 - (b) measuring or detecting the amount of the isotopic Rb^+ present in the liposomes or the cells; and
 - (c) comparing the isotopic Rb^+ present in the liposomes or cells measured or detected with measured or detected isotopic Rb^+ obtained by contacting liposomes containing, or cells containing or expressing, ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ with HIF in the presence of a compound comprising isotopic Rb^+ , under comparable conditions, thereby determining whether the test substance exhibits HIF inhibitory activity.
2. (Original) The method of Claim 1, further comprising the step of contacting ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ with HIF under suitable conditions in an aqueous medium for ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity, wherein said activity is measured or detected, thereby providing the measurement or detection to be compared with the test substance.

3. (Original) The method of Claim 1, wherein the ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase is obtained by substituting one or both border amino acid residues of the H1-H2 extracellular domain of α subunit of an ouabain-sensitive $\text{Na}^+ \text{K}^+$ -ATPase with a charged amino acid residue.
4. (Original) The method of Claim 1, wherein the isotopic Rb^+ is $^{86}\text{Rb}^+$.
5. (Original) The method of Claim 1, wherein the isotopic Rb^+ present in the liposomes containing the ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase is separated from the unincorporated or liberated compound comprising isotopic Rb^+ by chromatography.
6. (Original) The method of Claim 1, wherein the isotopic Rb^+ present in the cells containing the ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase is separated from the unincorporated or liberated compound comprising isotopic Rb^+ by centrifugation through an oil layer.
7. (Original) The method of Claim 6, wherein the oil layer is a phthalate oil layer.
8. (Original) The method of Claim 4, wherein the amount of $^{86}\text{Rb}^+$ present in the liposomes or the cells containing the ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase is measured by a gamma counter.
9. (Original) The method of Claim 1, wherein the ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase is isolated from a target cell.
10. (Original) The method of Claim 9, wherein the target cell is selected from the group consisting of a kidney cell, a heart cell, a pineal gland cell, a skeletal muscle cell, a retina horizontal cell, a retina Muller cell, a brain cortical astrocyte, a cerebellar granule neuron, a cortical neuron and a Hippocampal neuron.

11. (Currently amended) The method of Claim 9, wherein the target cell is ~~derived~~ obtained from a patient exhibiting a disease state that is related to dysfunction of the ouabain-resistant $\text{Na}^+ \text{K}^+ \text{-ATPase}$ in said target cell, thereby determining whether said target cell will respond to the test substance.
12. (Original) The method of Claim 11, wherein the disease state is selected from the group consisting of a cardiac malfunction in which a treatment to produce a positive inotropic effect is desired or needed, an edematous disorder and hypotension.
13. (Original) The method of Claim 12, wherein the cardiac malfunction is selected from the group consisting of congestive heart failure, paroxysmal atrial tachycardia and atrial fibrillation.
14. (Original) The method of Claim 12, wherein the edematous disorder is selected from the group consisting of congestive heart failure, cirrhosis of the liver and nephrotic syndrome.
15. (Original) The method of Claim 1, wherein the ouabain-resistant $\text{Na}^+ \text{K}^+ \text{-ATPase}$ is selected from the group consisting of a rodent, toad and butterfly $\gamma 1 \text{ Na}^+ \text{K}^+ \text{-ATPase}$.
16. (Original) The method of Claim 15, wherein the ouabain-resistant $\text{Na}^+ \text{K}^+ \text{-ATPase}$ is a rat kidney $\alpha 1$ ouabain-resistant $\text{Na}^+ \text{K}^+ \text{-ATPase}$.
17. (Original) The method of Claim 15, wherein the ouabain-resistant $\text{Na}^+ \text{K}^+ \text{-ATPase}$ is a *Bufo marinus* $\alpha 1$ ouabain-resistant $\text{Na}^+ \text{K}^+ \text{-ATPase}$.
18. (Original) The method of Claim 15, wherein the ouabain-resistant $\text{Na}^+ \text{K}^+ \text{-ATPase}$ is a *Danaus plexippus* $\alpha 1 \text{ Na}^+ \text{K}^+ \text{-ATPase}$.

19. (Original) The method of Claim 1, wherein the HIF is isolated from bovine hypothalamus.
20. (Withdrawn-Currently amended) A method for screening a test substance ~~other than ouabain~~, for ~~HIF-like~~ hypothalamic inhibitory factor (HIF) inhibitory activity of an ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$, comprising the steps of:
- (a) contacting the ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ with the test substance under suitable conditions for measuring or detecting ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity;
 - (b) measuring or detecting ATPase activity of the test substance; and
 - (c) comparing the activity of the ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ measured or detected in step (b) with the ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity of HIF under comparable conditions, thereby determining whether the test substance exhibits ~~HIF-like~~ inhibitory activity,
- wherein the ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ is obtained by substituting one or both border amino acid residues of the H1-H2 extracellular domain of α subunit of an ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ with a charged amino acid residue.
21. (Withdrawn-Currently amended) A method for screening a test substance ~~other than ouabain~~, for ~~HIF-like~~ hypothalamic inhibitory factor (HIF) inhibitory activity of an ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$, comprising the steps of:
- (a) contacting the ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ with the test substance under suitable conditions for measuring or detecting ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity;
 - (b) measuring or detecting ATPase activity of the test substance; and
 - (c) comparing the activity of the ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ measured or detected in step (b) with the ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity of HIF

under comparable conditions, thereby determining whether the test substance exhibits HIF-like inhibitory activity,

wherein the target cell is ~~derived~~ obtained from a patient exhibiting a disease state that is related to dysfunction of the ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase in said target cell, thereby determining whether said target cell will respond to the test substance.

22. (Withdrawn) The method of Claim 21, wherein the disease state is selected from the group consisting of a cardiac malfunction in which a treatment to produce a positive inotropic effect is desired or needed, an edematous disorder and hypotension.
23. (Withdrawn) The method of Claim 22, wherein the cardiac malfunction is selected from the group consisting of congestive heart failure, paroxysmal atrial tachycardia and atrial fibrillation.
24. (Withdrawn) The method of Claim 22, wherein the edematous disorder is selected from the group consisting of congestive heart failure, cirrhosis of the liver and nephrotic syndrome.
25. (Withdrawn-Currently amended) A method for screening a test substance for ~~HIF-like~~ hypothalamic inhibitory factor (HIF) inhibitory activity of an ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase, comprising the steps of:
 - (a) contacting the ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase with the test substance in the presence of ATP, wherein the terminal phosphate (P_3) is labeled, under suitable conditions for measuring or detecting liberated labeled P_3 ;
 - (b) measuring or detecting liberated labeled P_3 ; and
 - (c) comparing the measured or detected liberated labeled P_3 with the measured or detected labeled P_3 that is liberated by contacting ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase with HIF in the presence of ATP, wherein the terminal phosphate

is labeled, under comparable conditions, thereby determining whether the test substance exhibits HIF-like inhibitory activity,

wherein the ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ is obtained by substituting one or both border amino acid residues of the H1-H2 extracellular domain of α subunit of an ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ with a charged amino acid residue.

26. (Withdrawn-Currently amended) A method for screening a test substance for ~~HIF-like~~ hypothalamic inhibitory factor (HIF) inhibitory activity of an ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$, comprising the steps of:
 - (a) contacting the ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ with the test substance in the presence of ATP, wherein the terminal phosphate (P_3) is labeled, under suitable conditions for measuring or detecting liberated labeled P_3 ;
 - (b) measuring or detecting liberated labeled P_3 ; and
 - (c) comparing the measured or detected liberated labeled P_3 with the measured or detected labeled P_3 that is liberated by contacting ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ with HIF in the presence of ATP, wherein the terminal phosphate is labeled, under comparable conditions, thereby determining whether the test substance exhibits HIF-like inhibitory activity,

wherein the target cell is ~~derived~~ obtained from a patient exhibiting a disease state that is related to dysfunction of the ouabain-resistant $\text{Na}^+\text{-K}^+\text{-ATPase}$ in said target cell, thereby determining whether said target cell will respond to the test substance.
27. (Withdrawn) The method of Claim 26, wherein the disease state is selected from the group consisting of a cardiac malfunction in which a treatment to produce a positive inotropic effect is desired or needed, an edematous disorder and hypotension.
28. (Withdrawn) The method of Claim 27, wherein the cardiac malfunction is selected from the group consisting of congestive heart failure, paroxysmal atrial tachycardia and atrial fibrillation.

29. (Withdrawn) The method of Claim 27, wherein the edematous disorder is selected from the group consisting of congestive heart failure, cirrhosis of the liver and nephrotic syndrome.
30. (Withdrawn-Currently amended) A kit, comprising:
- (a) an isolated ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase, or reconstituted liposomes or cells containing an ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase;
 - (b) ~~HIF~~ hypothalamic inhibitory factor (HIF);
 - (c) a non-ATP substrate of said isolated ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase;
 - (d) ATP or labeled ATP; and
 - (e) a compound containing isotopic Rb^+ .
31. (Withdrawn-Currently amended) A kit comprising:
- (a) an isolated ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase;
 - (b) ATP in which the P_3 is labeled; and
 - (c) ~~HIF~~ hypothalamic inhibitory factor (HIF).
32. (Currently amended) A kit, comprising:
- (a) reconstituted liposomes containing, or cells containing or expressing, an ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase;
 - (b) a compound containing isotopic Rb^+ ; and
 - (c) hypothalamic inhibitory factor (HIF) HIF.
33. (Withdrawn) A biologically active substance identified by the method of Claim 1.
34. (Withdrawn) A method of treating or preventing a disease or disorder which comprises administering, to a subject to which such treatment or prevention is needed, an effective

amount of a biologically active substance identified by the assay of Claim 1, as exhibiting HIF inhibitory activity.

35. (Withdrawn) The method of Claim 34, wherein the disease or disorder is selected from the group consisting of a cardiac malfunction in which a treatment to produce a positive inotropic effect is desired or needed, an edematous disorder and hypotension.
36. (Withdrawn-Currently amended) A method of treating or preventing a disease or disorder which comprises administering, to a subject to which such treatment or prevention is needed, an effective amount of a biologically active substance identified by a method comprising the steps of:
- (a) contacting the ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase with the test substance under suitable conditions for measuring or detecting ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase activity;
 - (b) measuring or detecting ATPase activity of the test substance; and
 - (c) comparing the activity of the ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase measured or detected in step (b) with the ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase activity of HIF under comparable conditions, thereby determining whether the test substance exhibits HIF-like inhibitory activity.
37. (Withdrawn-Currently amended) A method of treating or preventing a disease or disorder which comprises administering, to a subject to which such treatment or prevention is needed, an effective amount of a biologically active substance identified by a method comprising the steps of:
- (a) contacting the ouabain-resistant $\text{Na}^+ \text{K}^+$ -ATPase with the test substance in the presence of ATP, wherein the terminal phosphate (P_3) is labeled, under suitable conditions for measuring or detecting liberated labeled P_3 ;
 - (b) measuring or detecting liberated labeled P_3 ; and

- (c) comparing the measured or detected liberated labeled p_3 with the measured or detected labeled P_3 that is liberated by contacting ouabain-resistant Na^+K^+ -ATPase with HIF in the presence of ATP, wherein the terminal phosphate is labeled, under comparable conditions, thereby determining whether the test substance exhibits HIF-like inhibitory activity.